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Inhibition of Moraxella-Acinetobacter Cells by Sodium Phosphates and Sodium Chloride

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ABSTRACT

The effect of sodium phosphates with and without NaCl, on the ability of unstressed and heat-stressed *Moraxella-Acinetobacter* (M-A) isolate number 7 cells to form colonies in plate count agar (PCA) was determined. The effectiveness, in order of decreasing ability, of phosphates to inhibit colony formation of unstressed M-A cells was as follows: sodium tripolyphosphate ($Na_5P_3O_{10}$; STPP), sodium pyrophosphate ($Na_4P_2O_7$; SPP), and sodium orthophosphate (Na_3PO_4; SP). Filter sterilized STPP was more inhibitory than when heated and inhibition was not related to pH. Certain combinations of NaCl and STPP were additive whereas combinations of NaCl and SPP exhibited a synergistic effect. Heat-stressed M-A cells were more sensitive to levels of NaCl (0.8%) than to levels of STPP (0.1%) or SPP (0.12%); all shown to have no effect on the number of colonies formed in PCA by unstressed cells.

INTRODUCTION

PHOSPHATES are used in various meat products to enhance the water-holding ability of meats (Ellinger, 1972). Their effect is greatly increased when used in combination with NaCl (Shults et al., 1972; Shults and Wierbicki, 1973, 1974). The polyphosphates also may prevent the discoloration of fresh and cured meat, improve texture, and prevent the development of off-flavors and off-odors (Ellinger, 1972). In foods such as red meats, poultry, and seafoods, the optimum level of polyphosphate addition for the above effects invariably falls within the range 0.35-0.5% retained polyphosphate (Mahon et al., 1971). Injection of a 5% polyphosphate solution into the breast of the carcasses of broiler chickens during commercial processing produced a net increase in the weight of carcasses subsequently chilled, frozen, thawed, or cooked (Grey et al., 1978). In all tests the injected birds were significantly more tender and juicier than broiler chickens without added polyphosphate (Griffiths and Wilkinson, 1978).

Commercial mixtures of phosphates inhibited the growth of certain bacteria in meat products. Dipping chicken carcasses in polyphosphate solutions reduced the number of bacteria (Spencer and Smith, 1962; Steinhauer and Banwart, 1964; Chen et al., 1973; Foster and Mead, 1976). Chen et al., (1973) reported that soaking chicken parts in 3% polyphosphate solution was effective in the control of gram-positive micrococci and staphylococci, but did not prevent the multiplication of gram-negative rods. Foster and Mead (1976) found that a final concentration of 0.35% polyphosphate in samples of minced chicken breast decreased the viability of samonellae during storage at $-2^{\circ}C$ or $-20^{\circ}C$ but not at $1^{\circ}C$ and $-5^{\circ}C$. Elliott and co-workers (1964) showed that nonfluorescent species of *Pseudomonas* were completely inhibited in the presence of polyphosphates in a broth medium, whereas fluorescent strains grew after a lag period. They speculated that inhibition was caused by the polyphosphates chelating metal ions essential to growth.

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Moraxella-Acinetobacter (M-A) cells have been isolated from irradiated meats (Tiwari and Maxcy, 1972) and under certain situations may be predominant in unirradiated beef and poultry (Seideman et al., 1976; Kraft et al., 1979; Juven and Gertshovki, 1976). Thermally stressed but viable M-A cells were intolerant of 0.8% NaCl in PCA and more sensitive to irradiation (Firstenberg-Eden et al., 1980a, b). The objective of this study was to determine the effect of phosphates, alone and in combination with sodium chloride, on the recovery (colony formation) of unstressed and heat-stressed cells of M-A.

MATERIALS & METHODS

Test organism

A radiation resistant culture of *Moraxella-Acinetobacter* (M-A) isolate number 7 was kindly provided by R.B. Maxcy, Univ. of Nebraska, Lincoln, Neb. This gram-negative coccobacilli was characterized by Welch and Maxcy (1975), and was found to be oxidase positive and saccharolytic. Such oxidase positive, saccharolytic strains showing *Moraxella* morphology and conforming to generic characteristics of both *Moraxella* and *Acinetobacter* are reported under "other *Moraxella*-like taxa" in the 8th edition of *Bergey's Manual of Determinative Bacteriology*. Isolate 7 cells divided in multiple planes and had a DNA base composition of 57.5%, unlike typical *Moraxella* or *Acinetobacter* species (Sanders and Maxcy, 1979). Since confusion still exists regarding the taxonomic position of isolate 7 we prefer to refer to it as M-A. The preparation of cultures for experiments and their maintenance was the same as previously described (Firstenberg-Eden et al., 1980a).

Media

Plate count agar (PCA; Difco Laboratories, Detroit, Mich.) was used as a reference for 100% survival. In those experiments concerned with pH, the pH of the medium was adjusted by adding 0.1N HCl or NaOH.

Chemicals

Sodium tripolyphosphate ($Na_5P_3O_{10}$;STPP), pyrophosphate ($Na_4P_2O_7$;SPP) orthophosphate (Na_3PO_4;SP) and sodium chloride (NaCl) were obtained from Fisher Scientific Company (Fair Lawn, N.J.) Unless otherwise specified the phosphates were sterilized by filtering through a 0.45 μm Millipore filter and added aseptically to autoclaved PCA, with or without NaCl, to yield final concentrations as shown in the individual experiments.

Heat-stressed cells

M-A cells inoculated into meat (about 10^8 cells/g were heat stressed at $70^{\circ}C$ for various time intervals as previously described (Firstenberg-Eden et al., 1980a). After heat treatment, the 20 g meat samples were aseptically introduced into a Waring Blendor jar with 180 ml of 0.1% peptone, and blended for 2 min at ambient temperature. The samples were diluted with 0.1% peptone, and pour plated with the following media; PCA, PCA + 0.8% NaCl with and without 0.03% STPP, PCA + 0.10% STPP and PCA + 0.12% SPP. After incubation at $32^{\circ}C$ for 5-10 days the creamy-pink colonies were counted.

RESULTS

Effect of pH on the growth of M-A cells

The same number of colonies was formed on PCA,

without added phosphates, over the pH range 5.5-8.0 (Fig. 1). At pH 5.0 and 9.0 there was no growth and at pH 8.5 only 2% of the original population formed colonies. The maximum pH change of PCA, as a result of the addition of 0.4% STPP or 0.5% SPP was from the usual 7.0 to 7.4 (data not shown). A change in pH of this magnitude would not affect the number of unstressed M-A cells capable of forming colonies on PCA.

Effect of STPP and SPP on colony formation

The tolerance of unstressed M-A cells to filter sterilized STPP is shown in Figure 2. A final concentration of 0.1% STPP in PCA had no effect on the number of colonies formed. However, the colonies were smaller than on PCA. Increasing the concentration above 0.1% progressively decreased the percentage of survivors. Less than 1.0% of the initial population was able to form colonies in PCA + 0.15% STPP during incubation for 10 days at 32°C. Heating autoclaved PCA with filter sterilized STPP at 70°C for 12 hr caused an apparent increase in the amount of STPP that the unstressed cells could tolerate (Fig. 2). After heating the PCA with STPP for 12 hr, 0.12% STPP had no effect while 0.35% was needed to completely prevent colony formation. A more extensive apparent increase in the concentration of STPP necessary to inhibit colony formation was noted when STPP was added to PCA and sterilized in an autoclave.

STPP was reported to be rapidly enzymatically hydrolyzed in meats to SPP and SP (Sutton, 1973). Therefore, it was desired to study the tolerance of unstressed cells of M-A to filter sterilized SPP and SP. Higher molar concentrations of SPP than of STPP were needed to inhibit colony formation (Fig. 3). Addition of 0.12% SPP to PCA (equivalent molar concentration of STPP is 0.16%) had no effect; the addition of 0.4% SPP (equivalent molar concentration of STPP is 0.55%) completely prevented colony formation.

Effect of combining STPP or SPP with NaCl on the colony formation of M-A cells

Formulated meats to be irradiated usually contain NaCl and STPP. The effects of different combinations of STPP and NaCl on the surviving fraction of unstressed cells of M-A are given in Figure 4. A minimum of 0.4% NaCl combined with 0.12% of filter sterilized STPP in PCA completely inhibited M-A cells from forming colonies (Fig. 4, □). The addition of 0.8% NaCl and 0.04% STPP resulted in the survival of 75% of the total population (Fig. 4, ○). When approximately half of the inhibition was caused by each one of the salts, the effect of STPP and NaCl was additive (a summation of the inhibition expected from each salt used singly). An example of the additive effect is as follows: The combination of 0.55% NaCl and 0.06% STPP in PCA resulted in the same surviving fraction of M-A cells as did either 1.1% NaCl or 0.12% STPP. An additive effect was also evident when 0.2% NaCl was combined with the maximal percent (0.1%) of STPP that did not inhibit M-A cells. However, this was not true when low concentrations (0.02-0.03%) of STPP were added to PCA containing 0.8% NaCl, the maximum tested concentration having no effect on the number of M-A cells forming colonies. Such combinations had no effect on the number of colonies formed by unstressed cells.

Rather than an additive effect, combinations of SPP and NaCl exhibited a synergistic effect (acting together the total effect was greater than the sum of their individual

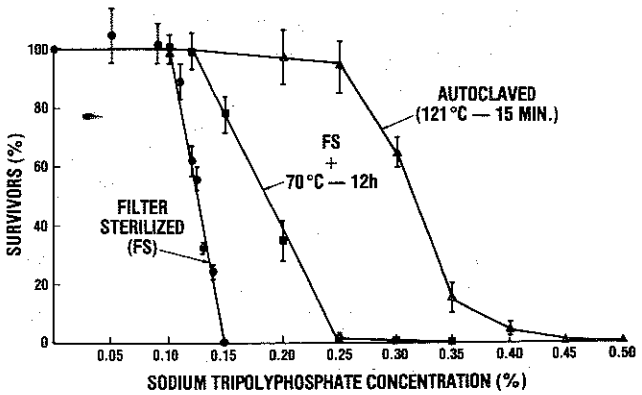

Fig. 2—Effect of concentration of unheated and heated sodium tripolyphosphate (STPP) on the recovery of M-A isolate 7 cells in plate count agar (PCA). Incubation at 32°C was for 7 days. Symbols; ●, filter sterilized STPP; ■, filter sterilized STPP, heated in autoclaved PCA for 12 hr at 70°C; ▲, PCA and STPP autoclaved (15 min at 121°C) together. The points and bars are as described in Fig. 1.

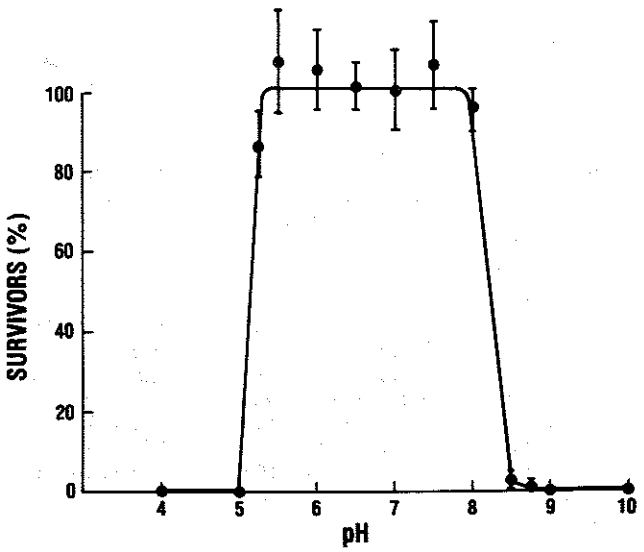

Fig. 1—Effect of pH of plate count agar on the recovery of M-A isolate 7 cells when incubated at 32°C for 7 days. The points show the average percent survivors (of 8-12 experiments) at each pH and the bars give the 95% confidence limits.

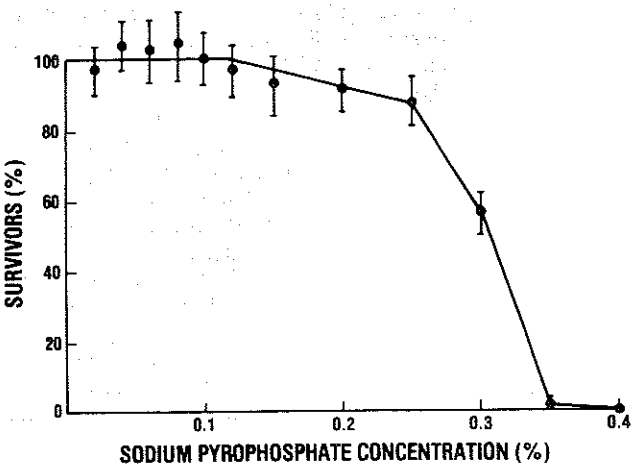

Fig. 3—Effect of concentration of filter sterilized sodium pyrophosphate (SPP) on the recovery of M-A isolate 7 cells in plate count agar. Incubation was at 32°C for 7 days. The points and bars are as described in Fig. 1.

effects). An example of this effect is as follows: the addition to PCA of either 1.1% NaCl or 0.3% SPP resulted in a surviving fraction of about 0.50 (Fig. 5). However, when the concentration of NaCl and SPP was halved and both were added to PCA, no colonies were formed by M-A cells. Furthermore, the addition of only 0.01% SPP together with 0.55% NaCl resulted in complete inhibition.

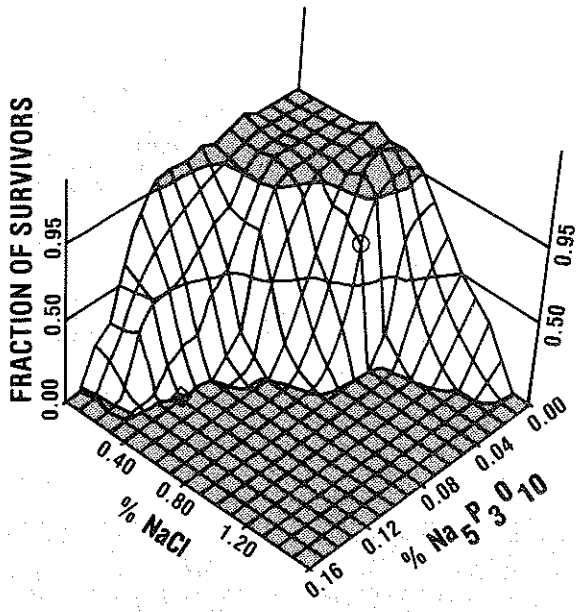

Fig. 4—Combined effect of sodium tripolyphosphate (STPP) and NaCl in plate count agar (PCA) on the recovery of M-A isolate 7 cells when incubated at 32°C for 7 days. Symbols: □, effect of addition of 0.4% NaCl together with 0.12 STPP to PCA on the fraction of M-A cells surviving; ○, effect of addition of 0.8% NaCl and 0.04% STPP to PCA on the fraction of M-A cells surviving. The lower and upper shaded areas show those concentrations of STPP and NaCl that resulted in less than 5% surviving cells and more than 95% surviving cells, respectively.

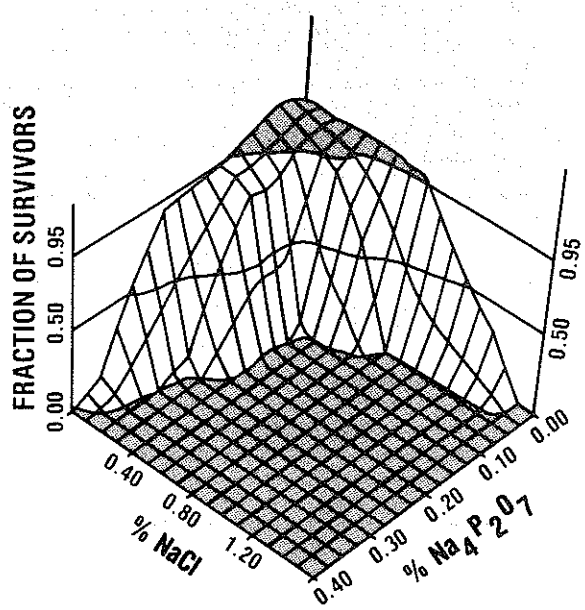

Fig. 5—Combined effect of sodium pyrophosphate (SPP) and NaCl in plate count agar on the recovery of M-A isolate 7 cells when incubated at 32°C for 7 days. With the exception that SPP was used instead of sodium tripolyphosphate the lower and upper shaded areas are as described in Fig. 4.

Effect of SP with or without NaCl on colony formation of M-A cells

The effect of filter sterilized SP on PCA on colony formation of M-A was much smaller than that of STPP or SPP (Table 1, Fig. 2 and 3). The addition of up to 0.5% SP did not affect significantly the number of colonies formed. However, if 0.5% SP were used in combination with 0.8% NaCl, more than 99.9% of the M-A cells failed to form colonies, and only about 9% formed colonies when 0.55% NaCl was added to 0.5% SP. M-A cells were much less sensitive to combinations of SP plus NaCl than to the combinations of NaCl with either STPP or SPP. Media containing 0.4% NaCl and 0.25% SP had no significant effect on colony formation while 0.4% NaCl in combinations with 0.2% SPP or 0.11% STPP completely inhibited colony formation.

Effect of polyphosphates alone and in combination with NaCl on heat-stressed cells

In these experiments ground meat, inoculated with M-A cells, was heated to an internal temperature of 70°C for various time intervals, cooled and the survivors were determined by plating on five different media (Fig. 6). Survival curves for the stressed cells recovered in the presence of 0.12% SPP and 0.10% STPP had shorter shoulders, and in the case of STPP, a steeper decline than those recovered on only PCA. However, heat-stressed cells were most sensitive to 0.80% NaCl with or without 0.03% STPP. The media with NaCl showed a rapid decline in uninjured cells during the first 6-7 min of heating, followed by a more moderate decline.

DISCUSSION

M-A CELLS (which are gram-negative coccobacilli) were found to be very sensitive to the two polyphosphates studied. Less than 0.35% was needed to inhibit colony formation by 90% of the population. Chen et al. (1973) showed that certain gram-negative rods (e.g. *Escherichia coli, Proteus mirabilis, Salmonella typhimurium* and *Pseudomonas aeruginosa*) tolerated concentrations of 1.0-6.0%.

It is clear from the present work and from others' work (Elliott et al., 1964; Kohl, 1971), that the effect of polyphosphates is not due to an increase in pH. The effect of polyphosphates as microbial inhibitors is believed to be due to their ability to chelate the essential metal ions (Elliott et al., 1964; Ellinger, 1972). However, factors other than metal chelation may be involved in microbial inhibition by polyphosphates (Ellinger, 1972).

To get the optimum benefit of the NaCl and STPP (e.g. enhanced fluid retention during heating and refrig-

Table 1—Effect of various concentrations of sodium orthophosphate (SP) with or without NaCl on the recovery of M-A isolate 7 cells on plate count agar (PCA) incubated at 32°C for 7 days

PCA		Recovery
SP (%)	NaCl (%)	(%)
0.50	0.00	98.0
0.70	0.00	85.3
1.00	0.00	65.2
0.25	0.40	98.9
0.20	0.80	13.9
0.30	0.40	85.2
0.50	0.55	9.1
0.50	0.80	0.05

eration, reduction in oxidative changes) the treated meat is usually held at −1 to 10°C (Mahon et al., 1971). During storage at 0°C for 8 hr the meat enzymes converted most of the STPP to SPP and SP (Sutton, 1973). Water retention increased during the enzymatic breakdown of STPP to SPP in minced, salted (2% NaCl) beef muscle (Neraal and Hamm, 1973). Heat treatment will result in a hydrolytic degradation of STPP to SPP and SP. At 70°C and 121°C in a pH 7.0-7.5 aqueous medium about 50 hr and 21 min, respectively, were required to hydrolyze half the STPP to SP (Van Wazer, 1971). Thus, as is evident in Figure 2, a given concentration of STPP is more effective as an inhibitor of M-A cells when filter sterilized than when heated at 70°C for 12 hr or autoclaved. The decreased effectiveness of autoclaved STPP may be due to both hydrolysis and reaction with ingredients of PCA. Both filter sterilized STPP and SPP are effective inhibitors of colony formation by M-A cells, whereas orthophosphate is ineffective in practical concentrations.

Salts (NaCl and STPP) in beef may play both a protective and inhibitory role. Firstenberg-Eden et al. (1980b) showed that fewer M-A cells were thermally inactivated or injured if NaCl and STPP were present in beef. However, heat-stressed M-A cells were very sensitive to 0.8% NaCl in PCA (Firstenberg-Eden et al., 1980a, b, and present study). The addition of 0.03% STPP did not cause any further reduction in stressed cells. The maximum concentration of STPP (0.1%) or SPP (0.12%) which had no effect on the recovery of unstressed cells had a less profound effect on the ability of heat stressed cells to form colonies than did 0.8% NaCl. It is evident from this study that unstressed or heat-stressed M-A cells would not be able to multiply in meats formulated with (wt/wt) 0.8% NaCl and concentrations of sodium phosphates >0.08% STPP, 0.05% SPP or 0.5% SP.

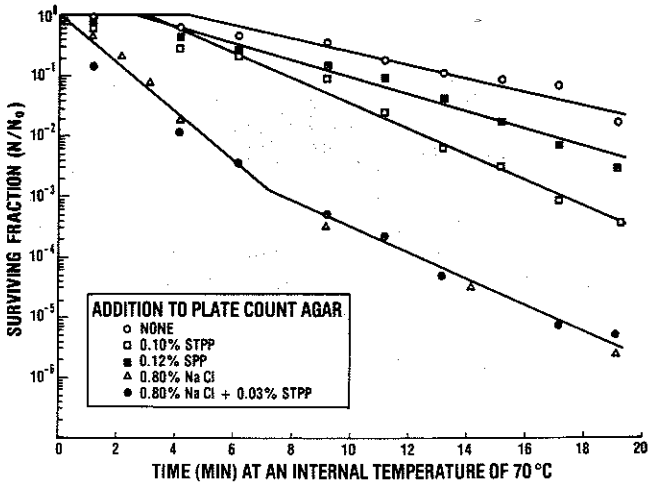

Fig. 6—Effect of sodium tripolyphosphate (STPP) and sodium pyrophosphate (SPP) and/or NaCl in plate count agar on the recovery of thermally stressed M-A isolate 7 cells at 32°C for 7 days. M-A cells were heated in meat at an internal temperature of 70°C for various time intervals, rapidly cooled, diluted and plated as shown. The time shown on the abscissa is the time after the meat reached an internal temperature of 70°C and was calculated as previously described (Firstenberg-Eden et al., 1980a).

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